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Analyzing Protein Changes in Guinea Pig Tissue Lysates Using Non-guinea Pig Specific Antibodies: Procedures for Western Blotting and Examples Using 16 Individual Antibodies for Common CNS Proteins

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14. ABSTRACT
Behavioral studies investigating chemical weapon nerve agent (CWNA) exposure often utilize guinea pigs because their levels of carboxylesterase enzymes are similar to humans (Maxwell et al. 1987; Shih and McDonough 1999; Shih and McDonough 2000; de Groot et al. 2001). However, the guinea pig model does present a significant problem when trying to correlate behavioral and protein changes due to the absence of guinea pig-specific antibodies. We have developed a procedure to determine the specificity of commercially available, non-guinea pig-specific antibodies in guinea pig lysates. Common Western blotting techniques were used to compare immunostaining patterns of tissue lysates between a known species, rat, and the guinea pig using antibodies to several common CNS proteins. Of the sixteen antibodies tested, nine revealed the exact same banding patterns as their rat lysate controls, five had similar banding patterns but required further characterization and two revealed no immunoreactivity. This report outlines the procedure for characterizing the immunoreactivity of these antibodies for use in guinea pig tissue lysates and identifies nine CNS proteins that can be labeled in this manner. This procedure should be of benefit to all investigators using the guinea pig behavioral model who wish also to investigate any underlying protein changes.

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Abstract:

Behavioral studies investigating chemical weapon nerve agent (CWNA) exposure often utilize guinea pigs because their levels of carboxylesterase enzymes are similar to humans (Maxwell et al. 1987; Shih and McDonough 1999; Shih and McDonough 2000; de Groot et al. 2001). However, the guinea pig model does present a significant problem when trying to correlate behavioral and protein changes due to the absence of guinea pig-specific antibodies. We have developed a procedure to determine the specificity of commercially available, non-guinea pig-specific antibodies in guinea pig lysates. Common Western blotting techniques were used to compare immunostaining patterns of tissue lysates between a known species, rat, and the guinea pig using antibodies to several common CNS proteins. Of the sixteen antibodies tested, nine revealed the exact same banding patterns as their rat lysate controls, five had similar banding patterns but required further characterization and two revealed no immunoreactivity. This report outlines the procedure for characterizing the immunoreactivity of these antibodies for use in guinea pig tissue lysates and identifies nine CNS proteins that can be labeled in this manner. This procedure should be of benefit to all investigators using the guinea pig behavioral model who wish also to investigate any underlying protein changes.

Introduction:

Many chemical weapon nerve agent (CWNA) studies utilize the guinea pig as the preferred species for animal modeling because levels of carboxylesterase enzymes in guinea pigs and humans are similar (Maxwell et al. 1987; Shih and McDonough 1999; Shih and McDonough 2000; de Groot et al. 2001). However, the guinea pig model does present a significant problem when trying to correlate CWNA-induced behavior changes to antibody-detectable protein changes within the organism. The absence of a comprehensive guinea pig genome sequence makes it difficult to determine homogeneity of proteins between species and increases the difficulty of making guinea pig-specific antibodies. In addition, biotechnology companies do not focus on guinea pig-specific products due to the prevalence of other rodents, such as mice and rats, in biomedical research. Therefore, most commercially available antibodies have been created based on protein tertiary structures from rat, mouse and human for which sequence information is readily available. Though it is common practice to use an antibody developed in one species to detect the same protein in another, such as a mouse antibody to detect a human protein, this is often done with access to the genetic sequences and/or protein structures of both species where compatibility and specificity can be better determined. It is often assumed that the protein sequence or tertiary structure used to create the antibody is conserved across species though this may not be the case, even with ubiquitous or highly conserved proteins. To complicate matters, antibodies are typically directed at 11-15 amino acid peptide sequences where a deviation of as few as 3 amino acids can greatly reduce immunoreactivity of the antibody. Therefore, to demonstrate that a species-specific antibody also labels the same protein in another species without the benefit of genetic or tertiary protein structure information, Western blots of the two species must be compared and reveal the correct predicted molecular weights. This technical report will illustrate a straightforward way to test and determine the specificity of commercially available antibodies in guinea pig lysates. In addition, this report will outline the immunolabeling procedures of several common CNS proteins in guinea pig tissue lysates. These protocols will help save time and money for researchers at the USAMRICD.

Materials and Methods:

Tissue Processing:
Neocortex, hippocampus, cerebellum, and cervical spinal cord tissues were rapidly excised from anesthetized, naïve Hartley guinea pigs and Sprague-Dawley rats. The samples were rapidly frozen in dry ice and then stored at -80º C. The tissue was homogenized in ice-cold triple detergent lysis buffer containing a Complete™ protease inhibitor cocktail (Roche Biochemicals; Indianapolis, IN) using a motorized pestle (Caframo; Wairton, ONT) and Tissue Tearer (Biospec; Racine, WI) on ice. Protein concentration was determined by bicinchoninic acid (BCA) micro protein assays (Pierce, Inc., Rockford, IL). Protein lysates were aliquoted and stored at -80º C to prevent repeated freeze-thaw cycles. All procedures were performed according to guidelines established by USAMRICD Institutional Animal Care and Use Committee (IACUC) and the National Institutes of Health (NIH).

Antibody Characterization using Western Blot:

A common Western blotting procedure was used with the Bio-Rad Ready Gel system with wet transfer (Bio-Rad; Hercules, CA). Forty micrograms of protein per well was loaded into 10-well 1.5 mm 6.5% or 15% acrylamide gels. The proteins were separated by SDS-PAGE gel electrophoresis at 150 V for 75 (6.5% gel) or 90 (15% gel) minutes. Protein was transferred to PVDF membranes (0.2 mm) at 100 V for 75 minutes. Membranes were blocked in 5% milk protein for 1 hour and then incubated in 3% milk/ primary antibody for 12 to 72 hours at 4º C. The membranes were then thoroughly rinsed in TBST and incubated in 3% milk/anti-rabbit (Sigma-Aldrich; St. Louis, MO) or anti-mouse (Zymed/Invitrogen; Carlsbad, CA) alkaline phosphatase-conjugated secondary antibody for 1 hour at room temperature with constant agitation at a concentration of 1:10,000. The PVDF membranes were developed using Enhanced Chemifluorescence reagents (ECF, Amersham; Arlington Heights, IL) and imaged using the STORM 860 (Molecular Dynamics; GE Healthcare, Piscataway, NJ).

Antibodies:

Antibodies used during this technique are summarized in Table 1. The antigen target (Protein), antibody production company (Company), catalog number (Cat. #), species the antibody was developed in (Species), antibody concentration ([Ab]) and incubation time to achieve optimal staining (Inc.Tm) are listed.

Results:

Detection of Structural Proteins:

Seven antibodies were tested for immunoreactivity to structural proteins in guinea pig tissue lysates and compared to rat tissue lysates in the cortex. These data have been summarized in Table 2.

The α-II-fodrin antibody revealed six distinct bands in both rat and guinea pig tissue lysates. These bands appeared at ~280, ~150, ~145, ~120, ~100 and ~80 kDa (Fig. 1A) in cortical lysates. This banding pattern is expected, as α-II-fodrin has a very characteristic and detectable breakdown pattern due to specific protease activity *in vivo* (Newcomb et al. 2000; Pike et al. 2001; Pike et al. 2004).

Microtubule associated protein 2 (MAP2) immunoreactivity was detected in both rat and guinea pig lysates. In rat, bands were consistently detected at ~175 and ~150 kDa (Fig. 1B, left lane). Guinea pig lysates revealed several bands with the highest immunoreactivity seen between ~125 and ~60 kDa. Bands at ~175 kDa, ~150 kDa and ~75 kDa were also present (Fig. 1B, middle lane). The three isoforms of MAP2 (A/B/C) have reported molecular weights of 280, 280 and 70 kDa (Izant and McIntosh 1980), a pattern that does not correspond well to the observed results in either species.

α-internexin immunoreactivity was detected as a prominent singlet at ~65 kDa in cortical rat tissue lysates (Fig. 1C, left lane) and at ~60 kDa in cortical guinea pig tissue lysates (Fig. 1C, middle lane). The reported molecular weight of α-internexin is 66-68 kDa (Pachter and Liem 1985).

Both neurofilament heavy (NFH) antibodies had similar immunoreactivity patterns in both rat and guinea pig lystaes. Four bands were present between ~260 kDa and ~150 kDa with an additional band at ~75 kDa in cortical guinea pig samples (Fig. 2A & B,

middle lanes). In cortical rat tissue lysates, three strongly immunoreactive bands, a doublet at ~220 kDa and a singlet at ~150 kDa (Fig. 2A & B, left lanes) were observed. Only the ~200 kDa band was shared in both species and is likely the hyperphosphorylated form of NFH (Shaw et al. 1984). The reported actual molecular weight of NFH is 280 – 220 kDa depending on phosphorylation state though it is often observed around 200 kDa (Sternberger and Sternberger 1983; Lee et al. 1986; Shaw et al. 2005).

Immunoreactivity for the neurofilament medium (NFM) and neurofilament light (NFL) antibodies was more precise than NFH in rat and guinea pig tissue lysates. NFM revealed a distinct singlet at ~145 kDa in both rat and guinea pig cortical lysates (Fig. 2C) and was in accordance with the reported molecular weight of 145 – 160 kDa (Schlaepfer and Freeman 1978; Hogue-Angeletti et al. 1982). A similar pattern was seen with NFL, where a singlet of ~70 kDa was observed (Fig. 2D) in rat and guinea pig lysates. The reported molecular weight of NFL is 68 kDa (Schlaepfer and Freeman 1978; Hogue-Angeletti et al. 1982).

Detection of Synaptic Proteins:

Two antibodies were tested for immunoreactivity to synapse-related proteins in rat and guinea pig tissue lysates. These data have been summarized in Table 2.

A single immunoreactive band for synaptophysin was detected at ~35 kDa (Wiedenmann and Franke 1985) in rat and guinea pig cortex (Fig. 3A). No immunoreactivity for the highly conserved synaptotagmin protein (Perin et al. 1991) was detected in any guinea pig tissue lysates, though a prominent band at ~75 kDa was detected in rat tissue lysates (Fig. 3B), close to the reported molecular weight of 65 kDa (Popoli and Paterno 1992).

Detection of Glutamate Receptor Proteins:

Four antibodies to AMPA glutamate receptor subunits and three antibodies to NMDA receptor subunits were tested for immunoreactivity in guinea pig tissue lysates and compared to rat tissue lysates. These data have been summarized in Table 2.

Immunoreactivity for glutamate receptor 1 subunit (GluR1) was light to undetectable in both rat and guinea pig lysates. When banding was visible, it appeared at ~100 kDa (Fig. 4A), consistent with the predicted molecular weight of 102-108 kDa (Sakimura et al. 1990). However, detection was sporadic and unreliable even at a very high antibody concentration. For glutamate receptor 2 and 3 subunits (GluR2/3), the dual detection antibody revealed a very prominent singlet in both rat and guinea pig lysates at ~100 kDa in cortex (Fig. 4B). This is consistent with the reported molecular weight of 105-107 kDa for both subunits (Sakimura et al. 1990). The antibody detecting only the glutamate receptor 3 subunit (GluR 3) revealed a single band at ~100 kDa (predicted at 105-107 kDa) (Hampson et al. 1992) in both rat and guinea pig cortical tissue lysates (Fig. 4C), though immunoreactivity was generally weaker than seen with the GluR2/3 antibody. No immunoreactivity for the glutamate receptor 4 subunit (GluR4) could be detected in any rat or guinea pig lysates (Fig. 4D).

NMDA receptor 1 (NMDAR1) subunit antibody revealed a lightly reactive singlet at ~170 kDa in both rat and guinea pig tissue lysates (Fig. 5A). However, the reported molecular weight of NMDAR1 is 116 kDa (Chazot et al. 1992). Immunoreactivity for the NMDA receptor 2a (NMDAR2a) subunit revealed a strong reactive band at ~170 kDa in

both rat and guinea pig lysates (Fig. 5B). This band is consistent with its reported molecular weight (Monyer et al. 1992; Joelson and Schwartz 1998). Other weaker bands were also present and can be seen in Figure 5.

A single, strong immunoreactive band for NMDAR2b was observed at ~180 kDa, the reported molecular weight (Monyer et al. 1992), in both rat and guinea pig tissue lysates (Fig. 5C). Two less prominent bands were observed at ~100 and ~60 kDa as well.

Amplification of Immunoreactivity:

An additional amplification step was attempted to increase the immunoreactivity of some of the antibodies that displayed less intense banding patterns. This step implemented a biotin/streptavidin secondary-to-tertiary complex that has been shown to amplify immunoreactive signals (Diamandis and Christopoulos 1991; Johnson et al. 2004). Because this step did not appreciably increase band intensity and caused the appearance of non-specific labeling, it was not included in further studies of these antibodies. It is thought that the non-specific bands were due to the reaction of naturally occurring biotin in the lysates with the added streptavidin.

Conclusions:

The guinea pig is the ideal species for behavioral CWNA research due to its having carboxylesterase enzyme levels similar to those in humans, though the primary set back in using this model beyond behavioral studies has been the lack of species-specific antibodies for immunohistochemical and protein quantification studies. This report has illustrated a way to test antibodies for compatibility with guinea pig tissue lysates using common Western blotting techniques. In addition, this report has characterized 16 antibodies for common CNS proteins in guinea pig cortical lysates.

Of the sixteen antibodies tested, nine (NFM, NFL, α-II-fodrin, synaptophysin, GluR1, GluR2/3, GluR3, NMDAR2a and NMDAR2b) revealed the exact same banding patterns as their rat lysate controls, indicating that the antibody was labeling the protein of interest and that no further characterization steps were necessary. However, in the case of the antibody for GluR1, further optimization will have to improve consistency and immunoreactive signal strength. An additional five (NMDAR1, NFH [Chemicon and Encor], MAP2 and α-internexin) would require further characterization. The final two antibodies (GluR4 and synaptotagmin) showed no immunoreactivity in guinea pig tissue lysates and were not pursued further.

The five antibodies requiring further characterization before using in guinea pig tissue lysates were for the proteins NMDAR1, NFH (both Chemicon and EnCor antibodies), MAP2 and α-internexin. Further characterization steps, such as protein sequencing, immunoprecipitation or further affinity purification, would likely have to be taken to confirm the immunoreactivity of the antibodies. Further characterization is necessary because of the appearance of extra bands or bands with a slightly different molecular weight.

For example, the NMDAR1 antibody detects a single band at ~160 kDa in both rat and guinea pig, though the reported molecular weight is 116 kDa. The difference in molecular weight cannot be attributed to species difference since both had the same banding pattern. It may be that the NMDAR1 subunit is complexed with other membrane proteins that are not removed during the tissue homogenization step since

the NMDAR1 antibody itself was characterized in synaptic fractions. Additionally, protein charge differences or phosphorylation state could make the protein run slower making it appear to have a higher molecular weight. However, this seems unlikely due to the reduced environment of the Western blot loading buffer.

NFH immunostaining shows five distinct bands in guinea pig compared to three in rat lysates. Of these bands, only one (~200 kDa) was shared in both species (Fig. 2A and B). NFH can become hyperphosphorylated, the prominent species in neuronal axons, and can be seen as a ~200 kDa band on Western blot (Shaw et al. 1984; Shaw et al. 2005). Additionally, the other observed bands could be breakdown products, less phosphorylated NFH species or possibly phosphoisotypes of NFM (Carden et al. 1985). The differences in the banding patterns likely arise from variations in the protein sequences for phosphorylation and proteolytic cleavage between species. However, without specific information on these variations, identifying the correct NFH band for comparative densitometry is problematic. Affinity purification has been shown to increase the specificity of these antibodies to the hyperphosphorylated ~200 kDa NFH species and reduce binding to other isoforms (Harris et al. 1991; Shaw et al. 2005) and would likely help in this situation as well. Similar structural properties may explain MAP2 banding patterns where two bands, corresponding at ~175 kDa and ~150 kDa, are shared by rat and guinea pig but with the most prominent bands occurring at much lower molecular weights in guinea pig lysates (Fig. 1B). Like NFH, MAP2 can also be phosphorylated (Sheterline 1977; Islam and Burns 1981) and is a target for proteolytic cleavage (Sloboda et al. 1976; Burns and Islam 1982) with species differences likely arising from species-specific phosporylation and cleavage sites.

Immunoreactivity for α-internexin revealed only one strong band in both rat and guinea pig lysates though the guinea pig variant was lighter by ~5 kDa (Fig. 1C). As with most filament proteins, α-internexin can be phosphorylated to increase the apparent molecular weight up to 4 kDa (Tanaka et al. 1993), which would account for the weight disparity. However, it seems highly unlikely that α-internexin in the two identically treated lysates would exist entirely in two different phosphorylation states. In addition, α-internexin tends to be highly conserved (>90%) between rats, mice and humans (Chan and Chiu 1996), so a dramatic difference in phosphorylation sites in the guinea pig would be unexpected. Though this antibody is almost assuredly specific for guinea pig α-internexin, protein sequence comparison should be done to verify this specificity.

In conclusion, this technical report outlines a straightforward protocol for testing commercially available antibodies for immunoreactivity in guinea pig lysates. Additionally, this procedure has already identified nine common CNS proteins that can be labeled in this manner along with optimization parameters. This procedure should be of benefit to all USAMRICD investigators using the guinea pig model who wish to investigate protein changes.

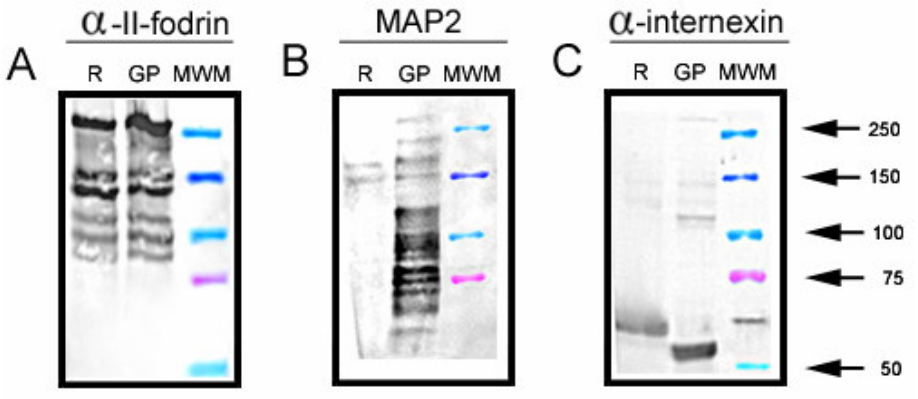

Figure 1: Representative Western blots of three CNS structural proteins in the rat and guinea pig cortex. Naïve rat and guinea pig cortical tissue homogenate proteins (40 µg) were separated using SDS-PAGE, immunoblotted with antibodies for α-II-fodrin (**A**), MAP2 (**B**) or α-internexin (**C**) and visualized as described in detail under Materials and Methods. Guinea pig immunolabeling patterns were then compared to the patterns in the rat. The antibody to α-II-fodrin revealed a similar banding pattern between rat (**A**, left lane) and guinea pig (**A**, middle lane) with prominent bands at 250, 150 and 145 kDa. MAP2 immunoreactivity was different between rat (**B**, left lane) and guinea pig (**B**, middle lane) but did share bands at 175 and 150 kDa. A single, strong band was present following α-internexin staining at ~65 kDa in rat (**C**, left lane) and at ~60 kDa in guinea pig (**C**, middle lane).

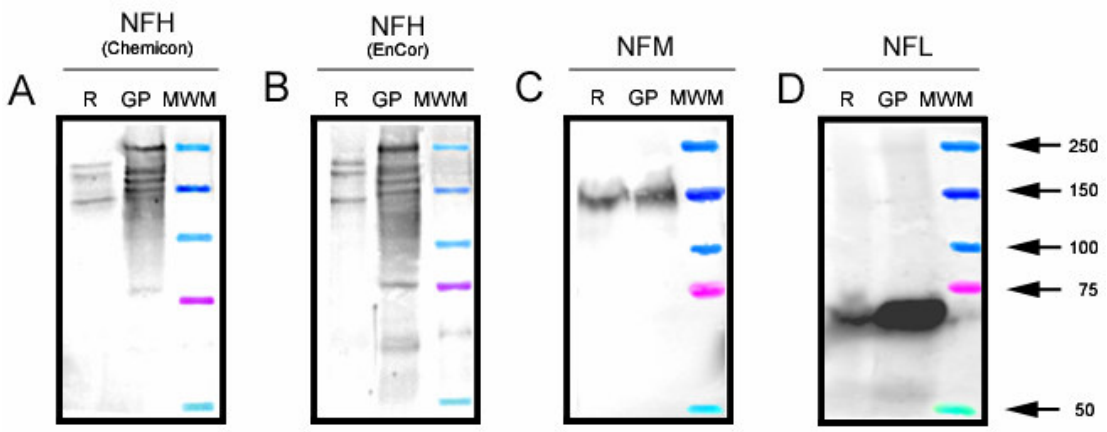

Figure 2: Representative Western blots of neurofilament proteins in the rat and guinea pig cortex. Naïve rat and guinea pig cortical tissue homogenate proteins (40 µg) were separated using SDS-PAGE, immunoblotted with antibodies for NFH (**A**, Chemicon; **B**, Encor), NFM (**C**) or NFL (**D**) and visualized as described in detail under Materials and Methods. Guinea pig immunolabeling patterns were then compared to the patterns in the rat. The antibodies to NFH (**A**, Chemicon and **B**, EnCor) had a very similar immunolabeling pattern to each other in both rat and guinea pig and will be discussed together. The banding pattern for NFH in guinea pig (**A & B**, middle lanes) was not similar to that seen in rat (**A & B**, left lanes). However, both rat and guinea pig labeled for a 200 kDa band, the major phosphoisotype of NFH. The antibody for NFM revealed a single ~145 kDa band in both guinea pig (**C**, middle lane) and rat (**C**, left lane) lysates. The antibody for NFL revealed a very strong single ~70 kDa band in guinea pig lysates (**C**, middle lane) and a less intense band in rat lysates (**C**, left lane).

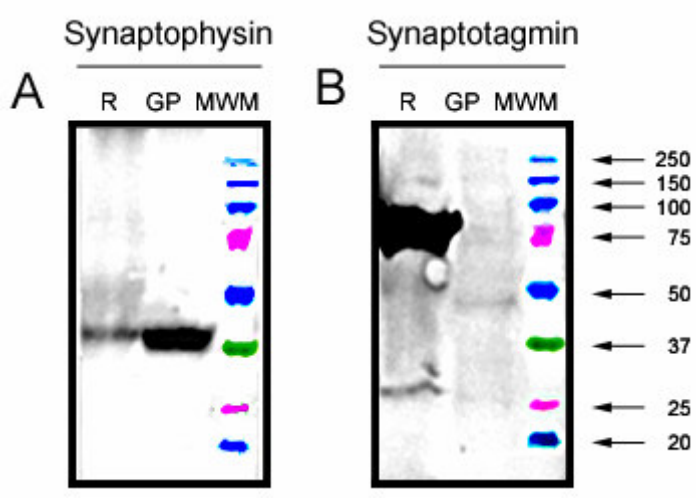

Figure 3: Representative Western blots of synaptic proteins in the rat and guinea pig cortex. Naïve rat and guinea pig cortical tissue homogenate proteins (40 µg) were separated using SDS-PAGE, immunoblotted with antibodies for synaptophysin (**A**) and synaptotagmin (**B**) and visualized as described in detail under Materials and Methods. Guinea pig immunolabeling patterns were then compared to the patterns in the rat. The antibody for synaptophysin revealed a very strong single ~35 kDa band in guinea pig lysates (**A**, middle lane) and a less intense band in rat lysates (**A**, left lane). The synaptotagmin antibody revealed a very strong single ~75 kDa band in rat lysates (**B**, left lane) but no immunoreactivity in guinea pig lysates (**B**, middle lane).

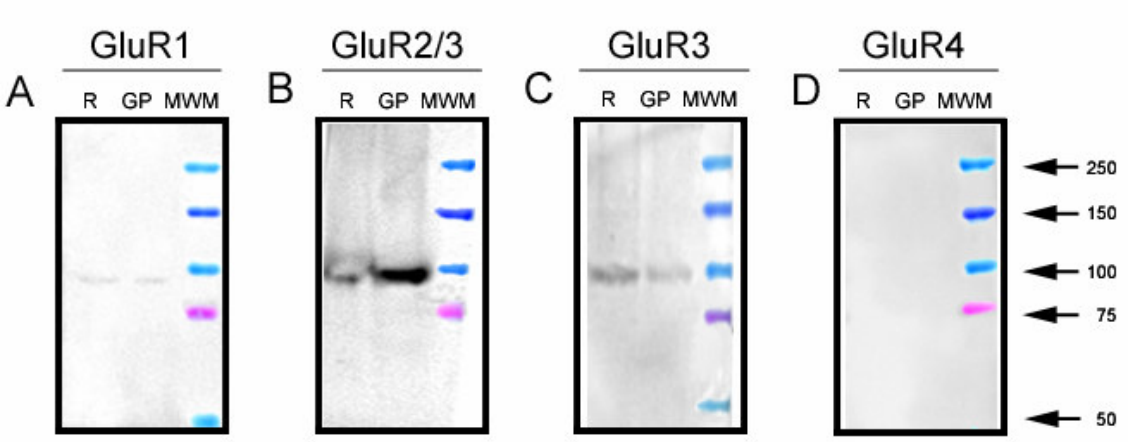

Figure 4: Representative Western blots of AMPA glutamate receptor subunit proteins in the rat and guinea pig cortex. Naïve rat and guinea pig cortical tissue homogenate proteins (40 µg) were separated using SDS-PAGE, immunoblotted with antibodies for GluR1 (**A**), GluR2/3 (**B**), GluR3 (**C**) or GluR4 (**D**) and visualized as described in detail under Materials and Methods. Guinea pig immunolabeling patterns were then compared to the patterns in the rat. The GluR1 antibody revealed a very weak, single band at ~100 kDa in both rat (**A**, left lane) and guinea pig lysates (**A**, middle lane). Labeling with this antibody was sporadic in all cortical tissues tested. Immunoreactivity for both GluR2/3 (**B**) and GluR3 (**C**) was detectable at ~100 kDa in both rat (left lane) and guinea pig tissue (middle lane) lysates. Immunoreactivity for GluR3 was weaker than that observed with GluR2/3. No immunoreactivity was observed in either rat or guinea pig lysates using the GluR4 antibody (**D**).

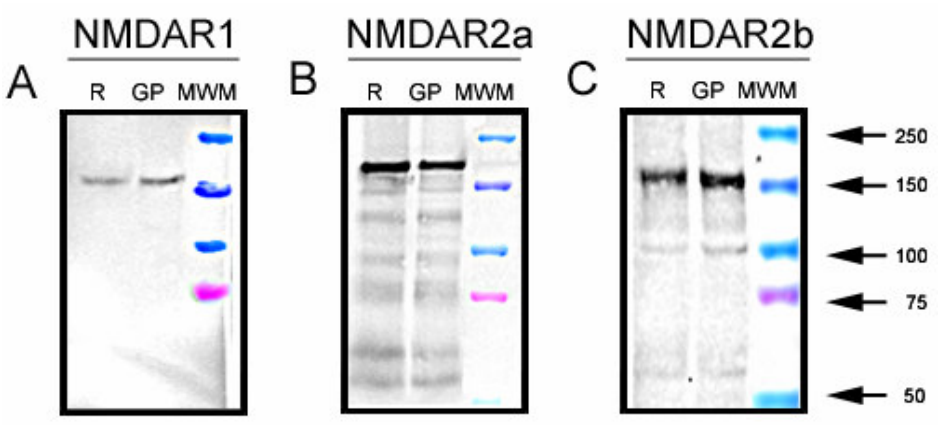

Figure 5: Representative Western blots of NMDA glutamate receptor subunit proteins in the rat and guinea pig cortex. Naïve rat and guinea pig cortical tissue homogenate proteins (40 µg) were separated using SDS-PAGE, immunoblotted with antibodies for NMDAR1 (**A**), NMDAR2a (**B**) and NMDAR2b (**C**) and visualized as described in detail under Materials and Methods. Guinea pig immunolabeling patterns were then compared to the patterns in the rat. The NMDAR1 antibody revealed a single band at ~170 kDa in both rat (**A**, left lane) and guinea pig lysates (**A**, middle lane). NMDAR2a (**B**) immunolabeling revealed a similar pattern in both rat and guinea pig lysates. This antibody detects a strong immunoreactive band at ~170 kDa and several weaker bands in both rat (**B**, left lane) and guinea pig (**B**, middle lane). Strong immunoreactivity for NMDAR2b (**C**) was observed in both rat (left lane) and guinea pig lysates (middle lane) at ~180 kDa with two lesser bands at ~100 and ~60 kDa in both species.

Antigen	Company	Cat. #	Species	[Ab]	Inc. Tm
Structural Proteins					
MAP2	Sigma-Aldrich	M1406	mouse	1:500	72 h
NFH	Sigma-Aldrich	N4142	rabbit	1:1000	12 h
NFH	EnCor Biotechnology	RPCA-NF-H	rabbit	1:10000	12 h
NFM	EnCor Biotechnology	RPCA-NF-M	rabbit	1:10000	12 h
NFL	EnCor Biotechnology	RPCA-NF-L	rabbit	1:2000	12 h
α-II-fodrin	Affiniti/Biomol International	FG6090	mouse	1:3000	12 h
α-internexin	EnCor Biotechnology	RPCA-α-int	rabbit	1:5000	12 h
Synaptic Proteins					
Synaptotagmin	Sigma-Aldrich	S2177	rabbit	1:500	72 h
Synaptophysin	Sigma-Aldrich	S5768	mouse	1:500	12 h
Receptor Proteins					
GluR1	Chemicon	AB1504	rabbit	1:100	72 h
GluR2/3	Chemicon	AB1506	rabbit	1:500	72 h
GluR3	Chemicon	MAb5416	rabbit	1:1000	72 h
GluR4	Chemicon	AB1508	rabbit	1:100	72 h
NMDAR1	Sigma-Aldrich	G8913	rabbit	1:500	72 h
NMDAR2a	Sigma-Aldrich	G9038	rabbit	1:500	72 h
NMDAR2b	Sigma-Aldrich	M265	rabbit	1:1000	72 h

Table 1: Summary of antibodies used. This table summarizes all antibodies used during this technique. The name of the antigen the antibody was produced against (Antigen), the company that made the antibody (Company), catalog number (Cat. #), the species the antibody was produced in (Species), the optimized concentration ([Ab]) and the optimized incubation time (Inc. Tm).

Protein	Predicted MW (kDa)	Observed MW (rat)	Observed MW (guinea pig)	Strength of Immuno-reactivity (guinea pig)
MAP2	280/ 280/ 70	175/ 150	280/ 220/ 175/ 150/ 125-60	Moderate
NFH	280-220	220/ 200/ 150	260/ 200/ 175/ 150/ 50	Strong
NFM	145-160	145	145	Moderate
NFL	68	70	70	Strong
α-II-fodrin	280/ 150/ 145/ 120	280/ 150/ 145/ 120/ 100/ 80	280/ 150/ 145/ 120/ 100/ 80	Strong
α-internexin	66-68	65	60	Strong
Synapto-tagmin	65	65	Absent	Absent
Synapto-physin	35	35	35	Strong
GluR1	102-108	100	100	Weak to Absent
GluR2/3	105-107	100	100	Strong
GluR3	105-107	100	100	Strong
GluR4	105-108	Absent	Absent	Absent
NMDAR1	116	110	110	Weak
NMDAR2a	170	170	170	Strong
NMDAR2b	180	180/ 100/ 60	180/ 100/ 60	Strong

Table 2: Antibody labeling and immunoreactivity data for guinea pig tissue lysates by brain region. This table summarizes the results from all Western blotting experiments for all antibodies in four brain regions. Weights for each immunolabeled band are reported in kilodaltons (kDa) under the columns Predicted MW, Observed MW (rat) and Observed MW (guinea pig). Immunoreactivity was labeled as either "Strong," "Moderate," "Weak," "Weak to Absent" and "Absent." "Strong" immunoreactivity is characterized as a dense (black) and wide band. "Moderate" immunoreactivity is characterized as a distinct, observable band with moderate density (dark gray). "Weak" immunoreactivity is characterized as an observable band over the background (light gray). "Weak to Absent" immunoreactivity is the same as "Weak" except the band density was so light that it was not always detectable in the blots. "Absent" is characterized as lack of any specific immunoreactivity.

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